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VITAMINS E AND A, CAROTENOIDS, AND FATTY ACIDS OF THE RAPTOR EGG YOLK

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ABSTRACT.—A captive population of falcons was fed a diet containing a known quantity of vitamin A (retinol) and vitamin E (α-tocopherol) for 6 wk prior to and during egg laying. Infertile eggs were analyzed for vitamin A, vitamin E, carotenoid, and fatty acid composition. Mean daily vitamin intake was 29 mg VitE (35IU) and 1157 μg VitA (3363IU). Adjusted mean egg yolk content for infertile, unincubated eggs was 314 μg/g α-tocopherol and 3.06 μg/g VitA. A distinctive feature of the raptor egg yolk is a very high proportion of arachidonic acid that is probably a reflection of their carnivorous diet. A small number of plasma samples were also available from egg-laying falcons. Mean plasma vitamin E was 32.2 μg/ml and plasma vitamin A 1.02 μg/ml.

KEY WORDS: *raptor nutrition; egg yolk; vitamins; fatty acids; plasma.*

Vitaminas E y A, carotenoides, y ácidos grasos de la yema de huevos de rapaces

RESUMEN.—Una población cautiva de halcones fue alimentada con una dieta que contenía una cantidad conocida de vitamina A (retinol) y vitamina E (α-tocoferol) por seis semanas antes y durante la postura de huevos. En los huevos infértiles fue analizada la composición de vitamina A, E carotenoides y ácidos grasos. La entrada media diaria de vitamina fue 29 mg VitE (35IU) y 1157 μg VitA (3363IU). La media ajustada para el contenido de yemas de huevos infértiles y no incubados fue 314 μg/g α-tocopherol y 3.06 μg/g VitA. Una característica distintiva de la yema de huevo de rapaces es una proporción muy alta de ácido araquidonico lo que probablemente es un reflejo de su dieta carnívora. Estuvieron disponibles tambien un pequeño numero de muestras de plasma de halcones durante la postura. El promedio de vitamina E en el plasma fue 32.2 μg/ml y 1.02 μg/ml de vitamina A.

[Traducción de César Márquez]

In recent years attention has focused on the breeding of certain raptor species in captivity as a means of conservation (Cade 1988, Fox and Fox 1993); one example is the Fiji Peregrine Falcon, *Falco peregrinus nesiotes* (D. Brimm pers. comm.). Productivity is dependent on good-quality eggs and without baseline data for raptor species it is difficult to assess egg quality in a breeding project. There are few data on the egg composition of wild or captive Falconiformes and virtually nothing is known about the fatty acid and the antioxidant profiles of the yolks of these species. Wild raptors have a predetermined clutch size and nutrients from the female, deposited in the egg prior to laying, provide all the necessary nutrition for the embryo to develop and for the chick to survive for a few days after hatching. Depending on the species, clutch size in Falconiformes is usually no more than five eggs. However in captivity, this number can be increased to as many as 14 eggs in one season by techniques of egg pulling and clutch pulling (Weaver and Cade 1991) and the requirement for nutrients is therefore much higher. Ideally, captive populations should be fed the same prey items that they would eat in the wild (Clum et al. 1997). Because this is often impractical, it is important to provide a varied, balanced diet with, if necessary, additional supplements of vitamins and minerals to ensure that the egg has sufficient nutrients needed to support successful development (Clum et al. 1997, Fox and Barton 2000).

In the yolk, vitamin E and carotenoids are lipid-soluble antioxidants which protect the developing embryo and chick against peroxidative damage,

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Table 1. Vitamin and water content of food items fed to captive raptors (Forbes and Flint 2000).

FOOD TYPE	SAMPLE SIZE	VITA IU/100 g DM	VITE IU/100 g DM	PERCENT WATER CONTENT
Whole day-old chick	200	497	40.7	76.1
De-yolked, day-old chick	200	363	21.4	78.5
VitE-enhanced quail	100	3633	10.1	66.6

regulate aspects of cell differentiation, and promote the function of the immune system (Surai and Speake 1998, Surai 1999). These are stored in the maternal liver and mobilized during the laying cycle. Studies on the Lesser Black-backed Gull (*Larus fuscus*) have shown that the concentrations of vitamin E and carotenoids in the yolk decrease with each egg laid as the maternal reserves become depleted (Royle et al. 1999). It is therefore possible that captive breeding programs which involve the extension of clutch sizes to increase productivity may result in eggs which are deficient in these antioxidants with potentially-harmful consequences for embryonic survival. There is also evidence that some captive-breeding projects for raptors and other birds may not be producing at maximum capability due to an inadequate dietary provision of vitamin E (Nichols and Montalli 1987, Dierenfeld et al. 1989). For example, captive Peregrine Falcons fed on whole quail (*Coturnix* spp.) achieved plasma vitamin E concentrations of only about 3 μg/ml compared with about 26 μg/ml in wild counterparts; injection or dietary supplementation of the quail with vitamin E was necessary for the captive peregrines to attain plasma vitamin E levels similar to those typical of the wild birds (Dierenfeld et al. 1989). Thus, supplementation of prey items with vitamin E may be needed to achieve optimal reproductive performance in captive raptors.

The polyunsaturated fatty acids of yolk lipids, especially the long-chain polyunsaturates arachidonic (20:4n-6) and docosahexaenoic (22:6n-3) acids, have vital roles in the functional development of certain embryonic tissues, particularly the brain and retina (Speake et al. 1998). For many avian and reptilian species, eggs produced in captivity often display markedly reduced levels of n-3 polyunsaturates, vitamin E, and carotenoids in comparison with eggs laid in the wild. Noble et al. (1996) and Speake et al. (1999a) suggested that these differences may be related to low hatchabilities in the captive situation.

The main aim of the present study was to evaluate the yolk concentrations of vitamins E and A and carotenoids in seven raptor species and one hybrid as part of a viable captive breeding program in which the female parents were fed on vitamin E-enriched quail and day-old chickens (*Gallus gallus*). Since there is currently very little information on the effects of a carnivorous diet on yolk lipids, the fatty acid composition of the yolk is also reported.

METHODS

Eggs were collected from captive raptors held at the Falcon Facility, U.K. Infertile, unincubated eggs were taken from imprint falcons that laid eggs prior to insemination. Other eggs analyzed were infertile, but had been incubated for 14 days, at which time infertility was confirmed. All the females were fed a diet of vitamin-E-supplemented quail, day-old cockerels, vitamin supplements (Nekton E and Nekton S—Günter Enderle, Pforzheim, Germany) and cod-liver oil. Nekton E (100 g) and Nekton S (100 g) mixed and dissolved in 1 litre water produces an injectable solution containing 5.66 mg/ml VitE (6.8 IU) and 229 μg/ml VitA (666 IU).

Vitamin content of food items (Table 1) was used to determine total daily vitamin E and vitamin A intake. Wet weights for quail, whole chick, and de-yolked chick were 200 g, 40 g, and 30 g, respectively. Over the winter from 1 August–1 February, female peregrines, Sakers (*F. cherrug*), Gyrfalcons (*F. rusticolus*), Gyrfalcon × Saker hybrids (*F. rusticolus* × *F. cherrug*), a Common Buzzard (*Buteo buteo*) and a Harris's Hawk (*Parabuteo unicinctus*) were fed 6 d of the week with up to eight de-yolked, day-old cockerels and 1 d with rabbit (*Oryctolagus cuniculus*). The estimated content of the daily food intake was 7 mg or 8 4 IU vitamin E and 45 μg or 131 IU vitamin A. New Zealand Falcons (*F. novaezeelandiae*) and a Barbary Falcon (*F. pelegrinoides*) were fed a smaller amount of the same diet, but the same concentration of supplements on a body mass basis.

The diet was changed on 1 February to three whole day-old chicks and half a quail enhanced with vitamin E, slightly less for the smaller Barbary and New Zealand falcons. Each chick was supplemented daily with an injectable solution of Nekton E and Nekton S containing 5.66 mg VitE/ml. Each chick was injected with 1 ml of the solution. Daily vitamin E intake from 1 February over 6 wk to the start of egg-laying and during egg-laying was calculated as 29 mg or 35 IU/day. Daily vitamin A intake was 1157 μg or 3363 IU, although from 1 March this was increased to ca. 1650 μg or 4800 IU vitamin A three

Table 2. Mean vitamin levels (μg/g) in raptor egg yolk from infertile, unincubated eggs and selected captive raptor species (SD in parentheses). *N* is the number of eggs.

SPECIES	*N*	α-TOCOPHEROL	γ-TOCOPHEROL	RETINOL	CAROTENOIDS
Saker	4	310 (53.8)	10.63 (4.7)	3.1 (1.0)	46.1 (25.8)
Peregrine	7	326 (72.0)	9.1 (1.3)	3.8 (0.5)	32.1 (14.3)
New Zealand Falcon	5	212 (67.3)	5.94 (0.8)	2.4 (0.4)	48.3
Barbary Falcon	1	247	10.78	3.3	—
Common Buzzard	4	443 (75.0)	16.84 (1.6)	2.49 (0.5)	53.8 (4.0)

times/wk when a cod-liver oil supplement was added. Weekly vitamin A intake was therefore about 27 000 IU.

Eggs were delivered to the Scottish Agricultural College, Department of Biochemistry and Nutrition where the yolk was separated from albumin. Vitamin A, vitamin E, carotenoid, and fatty acid levels were determined. Eggs were analyzed from eight peregrines, four Sakers, two Gyrfalcons, four New Zealand Falcons, one Harris's Hawk, one Common Buzzard, one Barbary Falcon and five Gyrfalcon × Saker hybrids. Vitamins A and E were determined by the method of McMurray et al. (1980). To determine carotenoid levels, 2 ml of tissue or yolk homogenate (20% in 0.01 M phosphate buffer, pH 7.4) were mixed with 2 ml ethanol. Hexane (5 ml) was then added and the mixture was shaken vigorously for 5 min. The hexane phase containing the carotenoids was separated by centrifugation and collected. The extraction was repeated twice more with 5 ml hexane. Hexane extracts were combined and carotenoids were determined from absorption at 446 nm. For lipid extraction, yolk samples were homogenized in an excess of chloroform:methanol (2·1, v/v) and extracts of total lipid were prepared. The extracts were subjected to thin layer chromatography on silica gel G using a solvent system of hexane:diethyl ether: formic acid (80:20:1, v/v) and the band corresponding to phospholipid was eluted from the silica with methanol. The total lipid extract as well as the isolated phospholipid fraction was transmethylated and the fatty acid composition was determined by gas-liquid chromatography (Speake et al. 1999a). The phospholipids are the major lipids found in cell membranes that are transferred from the yolk to the chick during embryogenesis.

During the course of routine veterinary investigations, plasma vitamin E and vitamin A levels were also measured in two Saker Falcons and one peregrine during the laying cycle.

RESULTS

For unincubated eggs from the falcons (Saker, peregrine, Gyrfalcon), mean α-tocopherol level was 320 μg/g (N = 11, SD = 60.6); mean α-tocopherol 11.24 μg/g (N = 11, SD = 3.2); mean retinol 3.55 μg/g (N = 11, SD = 0.77); mean carotenoids 36.7 μg/g (N = 9; SD = 17.5; Table 2). Using the peregrine data of seven incubated (Table 3) and seven non-incubated eggs (Table 2), incubating eggs significantly reduces the levels of α-tocopherol (t_{12} = 3.25, $P < 0.01$) and retinol (t_{12} = 5.71, $P < 0.01$).

Fatty acid composition of the raptor egg yolk included saturates (16:0 and 18:0), monounsaturates (16:1n-7, 18:1n-9, and 18:1n-7) and polyunsaturates (18:2n-6, 20:4n-6, and 22:6n-3). The fatty acid profiles of eggs from different raptors were similar (Table 4, 5) and only buzzard egg yolk composition had distinctive features, including the highest proportion of linoleic acid (18:2n-6) and lowest proportions of 16:1n-7 and 18:1n-7 acids compared to other raptor eggs. As with the total lipid, the phospholipid fraction of buzzard eggs was characterized by the highest proportion of 18:2n-6 and the lowest proportions of monounsaturated fatty acids compared to the other raptor species studied. A notable feature of the fatty acid profiles of total lipid and phospholipid was the very high proportion of arachidonic acid.

Table 3. Mean vitamin levels (μg/g) in raptor egg yolk from infertile eggs artificially incubated for 14 days (SD in parentheses). *N* is the number of eggs.

SPECIES	*N*	α-TOCOPHEROL	γ-TOCOPHEROL	RETINOL	CAROTENOIDS
Saker	17	290 (61.3)	7.9 (1.9)	2.24 (0.7)	40.0 (8.18)
Peregrine	7	261 (56.4)	8.84 (2.2)	2.15 (0.5)	37.0 (9.7)
Gyrfalcon	2	291	6.4	3.8	38.8
New Zealand Falcon	2	174	5.07	1.85	38.1
Gyr/Saker	5	234 (81)	6.2 (3.0)	2.7 (1.1)	37.1 (16.3)
Harris's Hawk	3	193 (25.1)	4.9 (0.2)	3.14 (0.9)	23.8 (7.1)

Table 4. Mean fatty acid composition of the total lipids extracted from egg yolk as a percentage of total extracted fatty acids (mean ± SD; *N* is number of eggs).

FATTY ACIDS	SAKER	PEREGRINE	BUZZARD	NEW ZEALAND FALCON	HYBRIDS	HARRIS'S HAWK
16:0	26.54 ± 0.25	27.14 ± 0.16	26.53 ± 0.16	26.58 ± 0.14	28.25 ± 0.49	26.2
16:1n-7	3.60 ± 0.14	3.36 ± 0.13	1.86 ± 0.12	3.03 ± 0.21	3.73 ± 0.45	2.71
18:0	6.61 ± 0.07	7.07 ± 0.13	7.37 ± 0.17	7.16 ± 0.11	6.45 ± 0.61	14.8
18:1n-9	40.28 ± 0.16	38.95 ± 0.27	37.24 ± 0.62	38.15 ± 0.73	39.42 ± 0.81	9.21
18:1n-7	3.27 ± 0.06	3.07 ± 0.10	2.32 ± 0.03	3.23 ± 0.05	3.16 ± 0.24	2.6
18:2n-6	9.22 ± 0.16	9.40 ± 0.34	13.95 ± 0.62	9.46 ± 0.83	9.32 ± 0.74	10.41
20:4n-6	5.72 ± 0.08	4.79 ± 0.65	6.23 ± 0.08	7.31 ± 0.13	5.39 ± 0.30	5.79
22:6n-3	1.65 ± 0.05	1.40 ± 0.17	1.82 ± 0.10	2.22 ± 0.07	—	2.73
N	20	14	4	4	5	2

From the three falcons where plasma vitamin levels were measured, mean α-tocopherol was 32.2 μg/ml and mean vitamin A was 1.02 μg/ml.

DISCUSSION

Sufficient eggs were available to provide summary statistics, but the number of individuals of each species limited interspecific comparisons. Incubation of infertile eggs decreases fat-soluble vitamin concentrations with vitamin A being the most sensitive to this process. Thus, for future analyses it is recommended to use fresh, unincubated eggs. In birds, the level of vitamin E in the egg yolk and embryonic tissues reflects its level in the food (Surai 1999). There are some species-specific differences in vitamin E accumulation and transfer to the egg yolk with chicken (*Gallus gallus domesticus*) being more effective compared to turkey (*Meleagris gallopavo*), duck (*Anas platyrhynchos*), or goose (*Anser anser*) (Surai et al. 1998). Raptor eggs contain a very high α-tocopherol concentration compared to other avian species (Dierenfeld et al. 1989), probably reflecting dietary vitamin E supplementation. It has been suggested that increased vitamin E supplementation may have a positive effect on falcon reproductive performances (Dierenfeld et al. 1989). In chickens, recent studies also show a positive effect of vitamin E on immune system development and a protective effect in stress conditions (Surai 1999). For certain species such as the Gyrfalcon which often show poor immune responses particularly in captivity or under stressful situations, adequate dietary vitamin E levels would be essential for good health.

Information is available on the concentration of vitamin E in the yolks of a range of avian species in the wild. These currently include the Lesser Black-backed Gull (*L. fuscus*) (Royle et al. 1999), the Canada Goose (*Branta canadensis*) (Speake et al. 1999a) and the Emperor Penguin (*Aptenodytes forsteri*) (Speake et al. 1999b) which all have vitamin E concentrations of about 80 μg/g fresh yolk.

Table 5. Mean fatty acid composition of the phospholipid fraction extracted from egg yolk as a percentage of total fatty acids (mean ± SD; *N* is number of eggs).

FATTY ACIDS	SAKER	PEREGRINE	BUZZARD	NEW ZEALAND FALCON	HYBRIDS	HARRIS'S HAWK
16:0	24.47 ± 0.21	23.39 ± 0.24	26.28 ± 0.11	24.99 ± 0.25	24.24 ± 0.73	25.91
16:1n-7	1.08 ± 0.05	0.91 ± 0.05	0.47 ± 0.03	0.74 ± 0.06	1.14 ± 0.14	0.78
18:0	18.88 ± 0.23	20.28 ± 0.18	19.39 ± 0.10	19.93 ± 0.16	18.51 ± 1.05	19.89
18:1n-9	19.20 ± 0.21	17.62 ± 0.48	10.99 ± 0.50	14.11 ± 0.59	18.97 ± 1.00	12.58
18:1n-7	2.50 ± 0.04	2.24 ± 0.05	1.86 ± 0.01	2.34 ± 0.04	2.39 ± 0.17	2.10
18:2n-6	6.98 ± 0.14	6.96 ± 0.45	12.51 ± 0.29	6.76 ± 0.34	7.54 ± 0.54	10.59
20:4n-6	19.10 ± 0.24	20.49 ± 0.26	21.30 ± 0.13	22.78 ± 0.15	19.16 ± 0.91	19.02
22:6n-3	4.31 ± 0.14	4.51 ± 0.18	4.13 ± 0.29	4.77 ± 0.10	4.63 ± 0.27	6.28
N	20	14	4	4	5	2

The raptor eggs of the present study contained vitamin E at concentrations averaging 330 μg/g and would therefore seem to be very well provisioned with this vitamin. Thus, feeding the female parent with quail and chickens enriched with vitamin E is a successful strategy for achieving high levels of this vitamin in the egg.

Despite the small sample size, the mean plasma vitamin E levels of 32.2 μg/ml are similar to the levels which the Peregrine Fund, Boise, ID U.S.A. measured in their captive peregrine population. They achieved these levels either by feeding quail that had been injected with vitamin E (220 IU/kg quail) or quail which had been raised on a diet containing 220 IU/kg feed. Wild peregrines on migration had plasma vitamin E levels of 26.3 μg/ml compared to captive peregrines at the Peregrine Fund with 3.4 μg/ml (Dierenfeld et al. 1989). Because migratory individuals probably have levels lower than breeding individuals, the levels measured in migratory falcons were taken as a minimum requirement for a healthy, captive-breeding population (Dierenfeld et al. 1989). From the three falcons in this study that plasma was taken, egg composition was also analyzed and the fertile eggs from these individuals were all viable and produced healthy offspring. There is no reason to assume that the infertile egg composition was any different.

The concentration of the carotenoids in the raptor yolks was similar to that in the first-laid eggs of *L. fuscus* (Royle et al. 1999) and higher than the values reported for *B. canadensis* (Speake et al. 1999a) and *A. forsteri* (Speake et al. 1999b). The vitamin A content of the raptor yolks was slightly less than the value reported for *L. fuscus* (Royle et al. 1999).

The salient feature of the fatty acid profiles of the raptor yolks is the very high proportion of arachidonic acid in the phospholipid fraction which is about five times higher than the values reported for the domestic chicken and for various wild granivorous and herbivorous birds (Speake and Thompson 1999). This may be a consequence of carnivory because the edible parts of many animals are a rich source of arachidonic acid (Phetteplace and Watkins 1989, Li et al. 1998). The proportion of docosahexaenoic acid in the phospholipid of the raptor yolks was similar to the values for eggs of the chicken and many wild birds, but less than the level reported for the piscivorous *A. forsteri* (Speake and Thompson 1999).

In conclusion, the prey items supplemented with vitamin E were a very effective means of fortifying the yolks of raptors with this antioxidant. Vitamin E deficiency reduces hatchability in the quail (Kling and Soares 1980) and has been identified as a cause of late embryo mortality in an established raptor breeding program (Dierenfeld et al. 1989). Achieving adequate levels of vitamin E, carotenoids, and polyunsaturated fatty acids in the yolk may be essential for the efficient reproduction of birds in captivity. Further studies should focus on analyzing egg composition of wild falcons because such a comparison would give important information for improvement of the falcon diets in captivity.

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